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Renal Handling of ^{125}I-Labelled Homologous Pancreatic Lipase and Amylase in the Rat[1])

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Summary: Experiments were carried out in vivo on rats and in vitro on tubular brush border vesicles in order to study the renal mechanisms of the elimination of pancreatic lipase and amylase from the circulation. Highly purified ^{125}I-labelled homologous lipase, amylase or ^{125}I-labelled di-iodo-tyrosine was injected intravenously in a single dose. The sieving coefficients of lipase and amylase were found to be 0.126 and 0.118 respectively. Less than 1% of the lipase activity but more than 10% of the radioactivity were found in the urine in the course of a 120 min experiment. In experiments with amylase, 16% of the enzyme activity and 19% of the radioactivity were present in the urine. Elimination of both enzymes showed first order kinetics and was of the same magnitude (17–24 min). The elimination curves of the radioactivity consisted of at least two components: a fast component immediately after the injection, which was identical with the decrease of the resp. enzyme activity; and a slow component (half-life 106 min), which in both cases proved to be identical with the half-life of di-iodo-tyrosine. In experiments with amylase, the excretion of protein-free ^{125}I-activity started later than with lipase. The radioactivity of ^{125}I-labelled lipase was taken up faster by brush-border-vesicles than that of ^{125}I-amylase. Liberation of protein-free ^{125}I-activity from both enzymes occurred at the same rate. At the end of the experiments the kidneys had no lipase or amylase activity, but they contained 5.4% (lipase), 3.8% (amylase) of the injected radioactivity. These results indicate that both pancreatic enzymes undergo renal filtration and reabsorption, followed by intrarenal degradation to amino acids. The rate of reabsorption of lipase substantially exceeds that of amylase. This is presumably due to the high affinity of lipase to the luminal membrane.

Introduction

In our previous report we were able to demonstrate that after intravenous injection the pancreatic enzymes lipase and amylase had nearly identical biological half-lives in the rat (1). Furthermore, evidence was presented that both enzymes were to a large extent eliminated from the circulation by the kidneys. Nevertheless, only amylase activity was found in the urine to any significant degree, although the renal clearance of lipase was about 18% higher than the clearance of amylase (1). This finding can only be explained by a higher rate of reabsorption and/or degradation of lipase than of amylase in the renal tubules. In order to elucidate the mechanism of renal elimination of pancreatic lipase and amylase from the blood, experiments were carried out in the rat using ^{125}I-labelled enzymes. This experimental set up has the advantage that some of the metabolites can be identified and their fate can be followed, even after degradation of the enzyme molecule.

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Materials and Methods

Experimental procedure

In vivo

Albino Wistar rats of 200–300 g body weight were used. The animals were narcotized with Nembutal® (40 mg/kg body weight) injected intraperitoneally. Subsequently a polyethylene catheter was placed into one of the external jugular veins. A continuous infusion of physiological saline was started (1.2 ml per hour) in order to secure sufficient diuresis as described previously (1). This was followed by opening the abdomen with a mid-line incision and cannulation of both ureters with polyethylene catheters (Intramedic® PE 10, Clay Adams, USA). The abdomen was closed by 2–3 sutures. After 30 min, highly purified homologous ^{125}I-labelled lipase, amylase (18.5–37 kBq ≙ 0.5 – 1.0 µCi, enzyme catalytic activity 50–200 U) or di-iodo-tyrosine (s. below), was administered in a single dose through the venous catheter. In several experiments this dose, as well as the subsequent saline infusion, also contained ^{14}C-labelled inulin or -tyrosine. Blood samples (60 µl) were collected from the tail every 10 min starting 5 min after injection. Sampling was carried out for 120 min. Experiments in which the sieving coefficient was to be determined were terminated 2.0 min after the injection (s. below).

The clearance of di-iodo-tyrosine was determined with the aid of ^{125}I-labelled di-iodo-tyrosine (s. below) together with ^{14}C-labelled *L*-tyrosine (Amersham-Buchler, FRG) by the classical infusion technique.

Urine samples were collected every 30 min from both kidneys. At the end of the experiment the abdomen was reopened and the animal was exsanguinated by drawing blood from the abdominal aorta. The kidneys were removed and weighed.

In vitro

For the determination of the rate of binding, uptake and degradation by the luminal surface of the tubular cells, brush border vesicles were prepared according to *Sastrasinh* et al. (2). The experiments were carried out as described by *Schöttke* et al. (3). The extent of intracellular degradation was determined by the rate of liberation of protein-free, non-precipitable ^{125}I-activity as described by *Emmanouel* et al. (4).

Analytical methods

Highly purified rat pancreatic lipase was prepared according to *Vandermeers & Christophe* (5). Lipase activity exceeded 750 kU/l. Measurement of the lipase activity of all samples was carried out using a Boehringer kit (Monotest Lipase® No 159 697) without modification (turbidimetric method). Amylase was purified using the affinity-chromatography method of *Burrill* et al. (6). For the determination of amylase activity the continuous UV test of Biomed, FRG employing maltotetraose as substrate (Monamyl-neu®) was used.

The blood content of the kidneys was determined as described previously (7).

^{125}I was purchased from Amersham-Buchler, FRG, [^{14}C]inulin from NEN, FRG. Iodination of the purified enzymes was carried out by the Chloramine-T method as described by *Bolton* (8). Loss of enzyme activity was smaller than 15 percent. Specific activity of the lipase preparation was 880 kBq/U ≙ 23.8 µCi/U, that of amylase 2.22 MBq/U ≙ 60 µCi/U. The iodinated sample was divided into suitable aliquots and kept at 2 °C. Immediately before use each aliquot was gel filtered on a PD-10 column packed with Sephadex G 25 M (bed volume 9 ml) and eluted with 9 g/l NaCl (sample size: 1 ml). Only the sample with the highest protein-bound activity was used for injection. – [^{125}I]tyrosine was prepared as follows: 100 µmol *L*-tyrosine (Serva, FRG), 150 µl glacial acetic acid, 80 µl concentrated HCl, 0.2 mmol NaI and 3.7 MBq ≙ 100 µCi ^{125}I-labelled NaI (Amersham-Buchler, FRG) were pipetted into an Eppendorf microtube. A single drop of hydrogen peroxide (30%) was added to the mixture and the tube was placed into a thermostat at 56 °C. After 10 h of oxidation and iodination the excess iodine was reconverted to NaI by adding excess of sodium thiosulphate to the tube (1 mol/l, 300 µl). Subsequently the content of the microtube was transferred to a screw-capped glass tube of 15 ml volume. It was then vigorously shaken with 5 ml followed by with 2.5 ml of diethyl ether for 5 min each time. This was done in order to remove any trace of free iodine. The ether phase was discarded. Di-iodo-tyrosine was removed from the water-phase by repeated extraction with methyl-acetate (5 ml, then 2.5 ml). The methyl-acetate fractions were combined and evaporated to complete dryness under a stream of air. The residue was dissolved in 5 ml of physiological saline containing 20 g/l of bovine albumin (*Cohn*-fraction V, – Serva, FRG). The purity of the preparation was checked after deproteinization by thin-layer chromatography on silica-gel plates (Merck No 60 F254, FRG) using butanol: glacial acetic acid: water 8 + 2 + 2 (by vol.) as developing solvent. Radioactivity was counted after removing the silica gel from the plate (s. below). Activity was found only at the position corresponding to di-iodo-tyrosine.

^{125}I radioactivity of blood, urine and tissue samples was counted in a well-type scintillation counter set exactly for 50% efficiency using ^{129}I as standard (Packard Instruments, USA). To secure equal counting geometry all samples counted were adjusted to 1 ml volume. An aliquot of the infusion served as reference. Urine samples as well as the last blood sample drawn from the aorta (20–100 µl) were separated on a PD-10 column packed with Sephadex G 25 M (s. above) eluted with 20 ml 9 g/l NaCl (sample size: 1 ml, 1–20 samples). Once again, the samples were counted. Aliquots of those samples with sizeable γ-radioactivity were further separated by thin-layer chromatography (s. above). ^{125}I-labelled lipase, 3-iodo-*L*-tyrosine and 3,5-di-iodo-*L*-tyrosine (Sigma Chemical Co., USA) were used as reference substances. After drying and identifying the non-radioactive reference substances with ninhydrin spray, the silica-gel coating of the plates was scratched off and counted (s. above). In all samples the recovery of γ-radioactivity applied was higher than 90 per cent. γ-Radioactivity was counted in a liquid scintillation spectrometer which allows the counting of double-labelled samples (Packard Tri-Carb C-300, USA).

Calculations

Serum half-life ($t_{1/2}$) for all substances tested was calculated from the blood samples by computing the least squares linear regression. For better understanding, the injected activity was set for 100% in every experiment. The renal clearance of ^{14}C-tyrosine and of ^{125}I-di-iodo-tyrosine was calculated using the classical clearance formula. Accumulation of radioactivity was expressed as a ratio of the activity of the kidney (counts per min, cpm), corrected for its blood volume, and of the activity of the blood at the end of the experiment (cpm/g kidney: cpm/ml blood). The glomerular sieving coefficient was determined by the fractional clearance method as described by *Maack* (9). Possible interference by metabolic processes was minimized by terminating the experiments within 2 min.

Statistical analyses were carried out using *Student*'s t test. Mean values ($\bar{x}$), standard deviation (S. D.) and the number of experiments (n) are given. Deviations were considered significant if the requirement $p \leq 0.05$ was fulfilled.

Results

Figure 1 shows the effect of diuresis on the excretion of lipase and amylase after a bolus injection. Urine samples contained only traces of lipase, irrespective of the rate of urine excretion (V). At the same time the excretion rate of amylase proved to be diuresis-dependent: amylase activity rose from 1% of the load at V = 1.9 µl/min linarly to 9.9% at V = 13.0 µl/min.

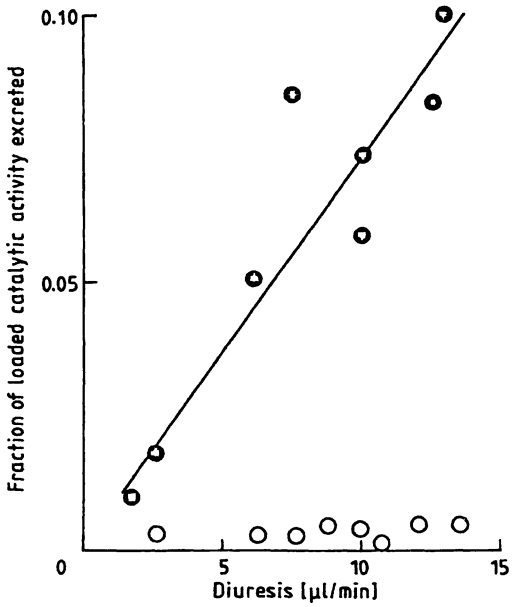

Fig. 1. Effect of diuresis on the excretion of lipase and amylase in the rat during 30 min following a bolus injection. Abscissa: diuresis (µl/min); ordinate: excreted enzyme catalytic activity (fraction of the load)
o = lipase;
• = amylase; y = 0.686x + 0.439; r = 0.91

The left and right panels of figure 2 show the decrease of catalytic and radioactivity of lipase and amylase after a single-dose application. The half-life of lipase was 18.1 ± 1.7 min (n = 7), that of amylase 21.6 ± 3.6 min (n = 6). The decrease in the radioactivity (right panel) shows curves which consisted of at least two different components. The fast component, calculated on the basis of the samples at 4–25 min by linear regression analysis, shows $t_{1/2}$ values of 17.7 ± 1.7 and 24.2 ± 2.4 min, respectively, which are identical to those above. The slow components (55–85 min) correspond to $t_{1/2}$ values of 106 ± 10.2 min and 104.2 ± 9.4 min, and they differ significantly from the previous data. The regression coefficient of the drawn linear regressions (r) was in no case lower than 0.99. Table 1 compiles data on the excretion of ^{125}I-labelled enzymes. In case of lipase, 0.21% of the injected catalytic activity, but 13.0% of the radioactivity were excreted within the observation period. In experiments with amylase, 15.9% of the catalytic and 18.8% of the radioactivity were found in the urine. Enzyme output reached its peak immediately after the injection (between 0 and 30 min) and declined afterwards. Excretion of ^{125}I-activity followed this pattern in experiments with amylase only. In contrast, in experiments with lipase, ^{125}I-activity was low in the first period and rose steadily afterwards.

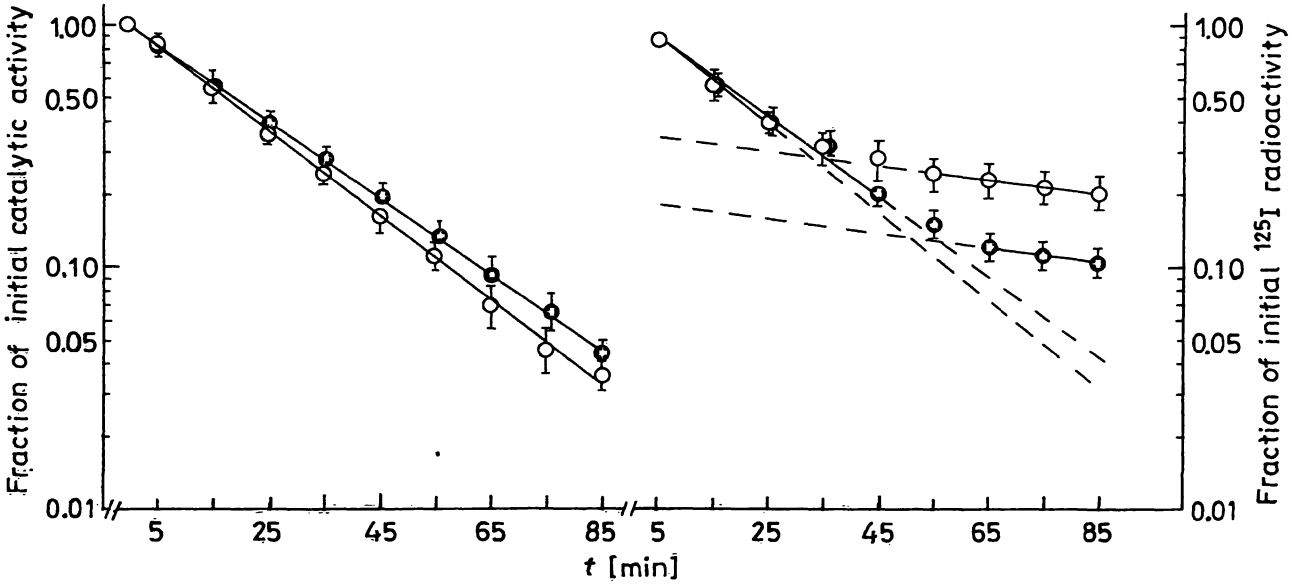

Fig. 2. Elimination rate of ^{125}I-labelled homologous lipase and amylase in the rat. Abscissa: time (min); ordinate: fraction of the initial activity in plasma;
Left panel: enzyme catalytic activity; least squares linear regression:
o = lipase; ln y = 0.0382x + 4.605; r = 0.99; $t_{1/2}$ = 18.1 min;
• = amylase; ln y = 0.0321x + 4.606; r = 0.99; $t_{1/2}$ = 21.6 min.
Right panel: ^{125}I-radioactivity; least squares linear regression:
o = lipase; fast component (5–25 min): ln y = 0.0392x + 4.610; r = 0.99; $t_{1/2}$ = 17.7 min;
slow component (55–85 min): ln y = 0.00659x + 3.559; r = 0.99; $t_{1/2}$ = 106 min.
• = amylase; fast component (5–25 min): ln y = 0.0387x + 4.590; r = 0.99;
slow component (55–85 min): ln y = 0.00655x + 4.595; r = 0.99; $t_{1/2}$ = 104 min.

Tab. 1. Recovery of catalytic activity and radioactivity in the urine after an intravenous injection of ^{125}I-labelled lipase or amylase.

Injected activity at 0 min: Lipase, 200 U, 1.1 × 10^6 counts/min ^{125}I-activity, (n = 8)
Amylase, 50 U, 1.1 × 10^6 counts/min ^{125}I-activity, (n = 8)

Sampling periods: 30 min

Sampling period (min)	Catalytic acitivity U, $\bar{x}$ ± S.D.		^{125}I-activity Counts/min $\bar{x}$ ± S.D. (% protein-bound)	
	Lipase	Amylase	Lipase	Amylase
U1 (0–30)	0.20 ± 0.1	5.9 ± 3.3	23400 ± 2080 (45.0)	89100 ± 8020 (88.2)
U 2 (30–60)	0.15 ± 0.05	1.0 ± 0.5	23800 ± 4440 (30.0)	47200 ± 5800 (58.7)
U 3 (60–90)	0.05 ± 0.03	0.8 ± 0.4	39400 ± 7430 (10.6)	35300 ± 8170 (32.8)
U 4 (90–120)	0.03 ± 0.01	0.2 ± 0.1	56300 ± 5300 (4.1)	35100 ± 7450 (19.0)
Σ: (0–120)	0.43 U	7.9 U	142900 counts/min	206700 counts/min
% of load:	0.21	15.9	13.0	18.8

Clearance of [^{14}C]inulin was 0.85 ± 0.02 ml/min × g kidney weight (n = 15). The clearance of [^{14}C]tyrosine equalled 0.013 ± 0.01 ml/min × g kidney weight (n = 26). [^{125}I]Di-iodo-tyrosine showed a clearance of 0.12 ± 0.015 ml/min × g kidney weight (n = 27). The half-life of serum [^{14}C]tyrosine was 292 ± 117 min (n = 5), that of di-iodo-tyrosine 109 ± 21.0 min (n = 10). Both clearance and $t_{1/2}$ of di-iodo-tyrosine exceeded those of tyrosine significantly.

Radioactivity in the kidneys at the end of the experiment with lipase constituted 5.4 ± 2.6% of the injected load and was 3.57 ± 0.97 times higher than the activity in the blood plasma (n = 26). Plasma contained at the end of the experiments 17.1 ± 3.4% of the injected ^{125}I-load. In experiments with amylase 3.8 ± 1.2% of the load was found in the kidneys. This activity was 1.6 ± 0.4 times higher than the activity of the plasma. The latter contained at the end of the experiments 13.6 ± 3.5% of the load (n = 12). The differences are significant.

The sieving coefficient of ^{125}I-labelled lipase was 0.126 ± 0.015 (n = 12), that of amylase 0.118 ± 0.012 (n = 12). The difference is significant.

In vitro studies showed that 2.1 ± 0.6% (n = 5) of the administered lipase and 0.67 ± 0.25% (n = 5) of the amylase were bound by brush border vesicles. The difference is significant. After 120 min incubation with brush-border vesicles 0.6 ± 0.2% (n = 6) of the ^{125}I-activity of lipase and 0.4 ± 0.2% (n = 5) of the amylase were found to be protein-free. The difference is not significant. 15 min incubation of ^{125}I-labelled lipase or ^{125}I-labelled amylase with kidney homogenates resulted in the liberation of 1.2 ± 0.3% (n = 6) or 1.5 ± 0.2% (n = 6) of the radioactivity, respectively. The difference is not significant.

Discussion

The data presented here show that injection of even large doses of lipase invariably leads to only a very small enzymuria. On the other hand, injection of amylase is followed by a significant and diuresis-dependent enzymuria (fig. 1). Since glomerular filtration of lipase exceeds that of amylase, we have to assume that lipase is reabsorbed preferentially by the renal tubules. This is most probably due to the 3–4 times higher affinity of lipase to the brush-border membrane of the renal tubules as compared to amylase; the more so, since there is no indication for intraluminal proteolysis of any of the enzymes. At the same time, the rate and result of intracellular metabolism are identical: the high ^{125}I-activity of the urine without enzymatic activity proved to be [^{125}I]di-iodo-tyrosine.

The short half-life of both lipase and amylase, the magnitude of their renal clearance (1) as well as the appearance of di-iodo-tyrosine a relatively short time after the injection of iodine-labelled preparations indicates that renal metabolism of both enzymes is fast and essentially complete.

Before discussing the results in detail some reservations have to be expressed concerning our experimental methods. This concerns in the first place the estimation of the sieving coefficient of lipase and amylase, since no perfect method for the determination of the sieving coefficient of a protein molecule exists (9). Thus, our calculations hold only if we assume that within the period of 2 min there were no differences in the handling of inulin and the enzyme by the kidney and that as far as the concentration of inulin and lipase are concerned, the blood drawn from the aorta reflects the blood in the glomerular capillaries. There

is no experimental proof for the correctness of this assumption. However, possible differences should be small. Furthermore it could be expected that the time span of 2 min was too short to allow accumulation and degradation of sizeable amounts of lipase and amylase in the tubular system (10). Furthermore, earlier data obtained in the isolated kidney support our present findings (11). Besides, these are in agreement with the predicted values for the sieving coefficients of a protein with M_r = 48 000 or 54 000 (12).

Taking this into consideration we interpret our results as follows: lipase or amylase injected intravenously was distributed primarily in the plasma volume. At the same time elimination was started both by extrarenal and by renal mechanisms, the latter being of greater importance (1). Renal elimination includes glomerular filtration as well as tubular reabsorption. The filtered amount constituted about 11–12% of the GFR as shown by the sieving coefficients. Since enzyme purity was high, the molar concentration of protein was in no case excessive. Under such conditions reabsorption of lipase was almost complete, as indicated by the very small lipase activity found in the urine (tab. 1). The appearance of lipase during the first period of the experiments can be best explained by a short-term overload exceeding the total capacity of the tubular system to reabsorb lipase; since the length and reabsorptive capacity of the renal tubules is not uniform, a certain spill-over may occur. This is due to nephrons with inherently small reabsorptive capacity (13, 14). The much lower affinity of amylase to the luminal membrane is well demonstrated by our experiments with brush-border-vesicles, and by the dependence of the urinary excretion on diuresis, thus indirectly on contact-time. In consequence almost 90% of the ^{125}I-activity excreted immediately following injection was protein-bound (versus 45% in experiments with lipase, see tab. 1).

Due to the short half-life of lipase and amylase (fig. 2, left panel), the enzyme load to the kidney decreased rapidly following injection. If we accept the hypothesis that the greatest part of reabsorbed enzymes was digested by the tubular system (1) and the metabolites (among them [^{125}I]di-iodo-tyrosine) were consequently released into the circulation and filtered in the kidney, the right panel of figure 2 can be explained: it shows the half-life of ^{125}I-labelled lipase or amylase (fast component) plus the half life of [^{125}I]di-iodo-tyrosine (slow component). Di-iodo-tyrosine was excreted to a substantial extent and was identified in the urine. As the result of this mechanism, very small enzyme-, but substantial ^{125}I-activity remained in the plasma by the end of the experiment.

The kidneys themselves did not accumulate active lipase or amylase and at 120 min contained only about five or four per cent, respectively, of the radioactivity. In the course of the experiments they continuously excreted increasing amounts of di-iodo-tyrosine (fig. 2, tab. 1). The latter finding can be explained by the increasing load to the tubular system, di-iodo-tyrosine being freely filterable and having a substantial clearance, which is about ten times higher than that of the physiological amino acid tyrosine (15).

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